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Carbon self-utilization may assist *Caulerpa taxifolia* invasion

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Running head: The role of carbon in *Caulerpa* invasion

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Abstract

Additions of ^{13}C-labeled macroalgae detritus (*Caulerpa taxifolia*) and seagrass detritus (*Zostera capricorni*) to a vegetated intertidal mudflat in subtropical Australia provided insight into the mechanisms and ecosystem effects of *C. taxifolia* invasions in seagrass beds. Despite the high lability typical of macroalgae, carbon from seagrass detritus was removed from sediments and transferred to benthic compartments (microphytobenthos, bacteria, mud whelks, and live *C. taxifolia* and *Z. capricorni*) at a faster rate than carbon from macroalgae detritus. This preference was more pronounced for live *Z. capricorni* than live *C. taxifolia*. Whereas rates of dissolved inorganic carbon (DIC) production and utilization were similar for seagrass and macroalgae detritus, higher fluxes of dissolved organic carbon (DOC) from macroalgae detritus early in the experiment reflect the lower utilization of this carbon pool. Although both seagrass and algae can use DOC as a source of carbon, caulerpenyne may comprise part of the DOC pool leached from *C. taxifolia* detritus during early degradation. This compound can have allelopathic effects on seagrass and may therefore be inaccessible to *Z. capricorni*. The availability of an additional carbon source for *C. taxifolia*, generated from its own detritus, represents a competitive advantage for this invasive macroalga over existing seagrass, particularly as seagrass productivity can be carbon limited. A similar mechanism may exist for other species of invasive macroalgae, particularly those that produce toxic secondary metabolites.

Introduction

The green macroalga *Caulerpa taxifolia* (Vahl) C. Agardh is among the world's 100 worst invasive species (Lowe et al. 2000), having successfully colonized areas of the Mediterranean Sea, California and southeastern Australia (Williams 2007), often outcompeting and replacing native macrophytes (de Villele and Verlaque 1995; Ceccherelli and Cinelli 1997). This poses a significant threat to biodiversity and ecosystem function, but the mechanisms that underlie the invasive success of *C. taxifolia* and its effects on invaded ecosystems remain largely unknown.

The effects of *C. taxifolia* invasion on habitat structure, consumer assemblages, trophic relationships and growth and productivity of native macrophytes are well-documented. *C. taxifolia* can outcompete and replace native seagrasses (de Villele and Verlaque 1995; Ceccherelli and Cinelli 1997), altering sediment biogeochemical conditions (Holmer et al. 2009) and processes (Eyre et al. in press). Fish species richness (York et al. 2006), abundance and biomass (Francour et al. 1995; Levi and Francour 2004) are often reduced in *C. taxifolia* beds, reflecting a preference of fish for seagrass (Burfeind et al. 2009), and *C. taxifolia* can alter assemblages of soft-sediment macroinvertebrates (Gribben and Wright 2006; McKinnon et al. 2009). Although *C. taxifolia* produces toxic secondary metabolites (primarily caulerpenyne) to discourage herbivory (Amade and Lemee 1998), there is evidence that it provides nutrition for some biota (Box et al. 2009; Casu et al. 2009), suggesting that invasion alters trophic relationships. A major influence on the differences in trophic relationships and ecosystem functioning observed in habitats invaded by *C. taxifolia* is likely to be the altered detritus input to sediments.

Sediment organic matter concentrations are similar in *C. taxifolia* meadows and seagrass beds (Holmer et al. 2009). However, fundamental differences in seagrass- and algae-derived detritus will be reflected in its processing and utilization. Whereas seagrass has a high carbon to nitrogen ratio (C:N) and a high content of refractory components such as

lignocellulose, macroalgae typically have a low C:N ratio and low fiber content (Bourges et al. 1996). Macroalgae is therefore usually degraded rapidly and is thought to offer a labile, accessible source of energy (carbon) for consumers (Mann 1988). Labile organic matter can also enhance degradation of existing refractory material by stimulating the sediment bacterial community (Frankignoulle et al. 1998). In the case of macroalgae of the *Caulerpa* genus, however, toxic caulerpenynes may be concentrated during degradation and/or transformed into more reactive products (Raniello et al. 2007), inhibiting its processing and utilization. Differences in the quality of *C. taxifolia* and seagrass detritus could affect the release of dissolved inorganic and organic carbon (DIC and DOC), influencing trophic transfer of carbon among heterotrophs and uptake by plants. Many seagrass species demonstrate increased growth following CO_2 enrichment, suggesting that carbon may limit their productivity (Abel 1984; Durako 1993; Invers et al. 2001). Altered carbon cycling following *C. taxifolia* invasion therefore has the potential to affect seagrass health and, concomitantly, associated biota.

Macroalgae is typically labile and rapidly degraded, but caulerpenyne in *C. taxifolia* detritus may limit its processing. As such, our hypothesis was that an input of *C. taxifolia* detritus may either enhance or limit carbon availability. To test this hypothesis we added ^{13}C-labeled macroalgae (*C. taxifolia*) and seagrass (*Zostera capricorni*) detritus to separate plots on a vegetated (*C. taxifolia* and *Z. capricorni*) intertidal mudflat and traced the transfer of carbon from detritus through benthic compartments (sediment organic matter, bacteria, microphytobenthos (MPB), fauna, live *Z. capricorni*, and live *C. taxifolia*). We also monitored the release of detritus-derived carbon from the sediment as fluxes of ^{13}C-labeled DOC ($DO^{13}C$) and DIC ($DI^{13}C$). By comparing the processing of seagrass- and macroalgae-derived carbon we aimed to gain insight into mechanisms that underlie the effects of *C. taxifolia* in invaded systems and, particularly, mechanisms that may assist its invasion into areas previously occupied by seagrass.

Methods

Study site

The study site was in the lower intertidal area of a mudflat in southern Moreton Bay, subtropical Australia (27°46'58.3"N, 153°23'00.2"E). *Zostera capricorni* (seagrass) and *Caulerpa taxifolia* (macroalga) were the dominant macrophytes and were exposed on most low tides. *C. taxifolia* is considered native to the area, but its range has expanded to include large areas that were formerly occupied by seagrass (Burfeind and Udy 2009). The site was net autotrophic (productivity to respiration ratio = 1.07 ± 0.09 (average for controls)) and surface sediments (< 1 cm depth) had an organic carbon content of 7888 ± 1154 mmol C m^{-2}.

Preparation of labeled detritus

Z. capricorni and *C. taxifolia* were collected adjacent to the study site, retaining sediment around roots and rhizoids, and were gently scraped clean of epiphytes. Approximately half of the aboveground material was retained for use as unlabeled detritus. The remaining *Z. capricorni* and *C. taxifolia* was transplanted into separate, oxygenated 40 L aquaria of sediment and site water maintained at in situ temperature (24°C). After 24 h, the water in each aquarium was replaced with 12 L of fresh site water containing 2 g $NaH^{13}CO_3$ (99% ^{13}C, Cambridge Isotope Laboratories). Aquaria were sealed with transparent PVC to minimize $^{13}CO_2$ loss and incubated under constant light to maximize macrophyte isotope enrichment. Frond samples were removed from each aquaria every 24 h to analyze ^{13}C incorporation. After 5 d, all aboveground material was removed, acid rinsed, scraped clean of epiphytes, cut into pieces (0.5 – 1.0 cm) and frozen for use as labeled detritus.

Detritus addition

In July 2007, at low tide, 12 plots 5 - 10 m apart were selected at the site. Three plots were allocated to each four treatments: 1) addition of ^{13}C-labeled *C. taxifolia* and unlabeled

Z. capricorni detritus (CT), 2) addition of ^{13}C-labeled *Z. capricorni* and unlabeled *C. taxifolia* (ZC), 3) procedural control (PC), and 4) control (C). Plots were marked by polyethylene frames (39 cm × 60 cm) inserted ~15 cm into the sediment, enclosing mixed live *Z. capricorni* and *C. taxifolia*. Approximately 14 g (wet weight) of *Z. capricorni* detritus (~ 49.5 mmol ^{13}C m^{-2}) and 18 g (wet weight) of *C. taxifolia* detritus (101.8 mmol ^{13}C m^{-2})was added to each CT and ZC plot. Detritus addition was intended to introduce the ^{13}C tracer without substantially altering sediment organic carbon content. Reflecting this, the added organic carbon represented 0.6% (*Z. capricorni*) and 1.3% (*C. taxifolia*) of the total sediment organic carbon within each plot. Detritus was buried under ~2 mm of surface sediment from outside the plots to minimize resuspension, while avoiding disturbance of sediment microhabitats. PC plots had surface sediments similarly reworked and were compared to C plots to test for procedural effects. C plots provided background values for excess ^{13}C calculations.

Sample collection

At 1 d after detritus addition, sediment was collected from each plot to determine the δ^{13}C of sediment organic matter and of biomarkers specific to bacteria and microphytobenthos (MPB). One sediment core (90 mm inner diameter (i.d.) × ~20 cm depth) was also collected from each plot in a Plexiglas core liner at 3, 6, 11, 19, and 29 d after detritus addition. Empty liners (90 mm i.d. × ~ 20 cm depth) were placed in holes left by core removal and filled with sediment to minimize site disturbance. Within 2 h of collection, cores were placed in incubation tanks (separate tanks for each treatment) of site water at in situ temperature (18 - 22°C) in direct natural sunlight. Magnetic stirrers circulated water at a rate just below the sediment re-suspension threshold. Cores were incubated for 24 h following a 24 h pre-incubation period.

Samples for analysis of DOC and DIC isotopes and concentrations were collected from cores via sampling ports in the lids just after dusk, just before the following dawn, then just

before the following dusk. Sample water was filtered (precombusted 47 mm GF/F), killed (200 μL 50:50 w:v $ZnCl_2$), sealed in 40 mL glass vials without headspace and refrigerated until analysis. Sample water was replaced, as it was withdrawn, from gravity-fed collapsible reservoirs of site water.

At the conclusion of incubations (5, 8, 13, 21, and 31 days after detritus addition), live aboveground *C. taxifolia* and *Z. capricorni* biomass was collected for $\delta^{13}C$ analysis and surface sediment (< 1 cm depth) was collected for $\delta^{13}C$ analysis of sediment organic matter and biomarkers specific to MPB and bacteria. Remaining sediment was sieved for fauna (1 mm mesh). Only one species (Australian mud whelk, *Velacumantus australis*) occurred across all treatments at most times and was analyzed for $\delta^{13}C$.

Stable isotope analysis

Prior to stable isotope analysis, macrophyte samples were cleaned of epiphytes, soft tissues of mud whelks were removed from shells, and sediment was acidified (1 mol L^{-1} HCl) to remove inorganic carbon. Samples were then lyophilized and homogenized. $\delta^{13}C$, %C, and %N were determined using a Thermo Finnigan Flash EA 1112 interfaced via a Thermo Conflo III with a Thermo Delta V Plus IRMS. Molar C:N ratios were calculated from %C and %N values.

Phospholipid biomarkers (PLFAs) to determine ^{13}C uptake into bacteria and MPB were extracted from lyophilized sediments, following addition of an internal standard (500 μL of 500 μg mL^{-1} tridecanoic acid, C_{13}), using a modified Bligh and Dyer method, as described by Oakes et al. (2010*a*). Concentrations and $\delta^{13}C$ values of PLFAs were determined using a Thermo Trace GC Ultra gas chromatograph with a non-polar 60 m HP5-MS column (J&W Scientific, 0.32 mm i.d., 0.25 μm film thickness), coupled with a Thermo Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) via a Thermo Conflo III interface (as described in Oakes et al. 2010*a*).

Concentrations and isotope ratios of DOC and DIC were measured via continuous flow wet-oxidation isotope ratio mass spectrometry (CF-WO-IRMS) using an Aurora 1030W TOC analyzer coupled to a Thermo Delta V Plus IRMS (Oakes et al. 2010*b*; Maher and Eyre 2011). Reproducibility for DOC and DIC, respectively, was ± 24 μmol L^{-1} and ± 33 μmol L^{-1} (concentrations) and ± 0.08‰ and ± 0.10‰ ($\delta^{13}C$).

Calculations

Total uptake (incorporation) of ^{13}C into sediment organic matter, bacteria, MPB, macrophytes and whelks (μmol ^{13}C m^{-2}) was calculated as the product of excess ^{13}C (fraction ^{13}C in labeled sample – fraction ^{13}C in control) and the mass of carbon in each compartment. Total C mass in sediment organic matter, macrophytes and whelks was determined using %C values and measured dry biomass per area. For bacteria and MPB, the peak area of the C_{13} internal standard was used to calculate concentrations of individual PLFAs based on their peak areas. Total bacterial biomass was determined using the biomarkers i15:0 and a15:0, and MPB biomass was determined using the algal biomarker 16:1(n-7). Calculations were done as described by Oakes et al. (2010*a*), except that the average fraction of MPB PLFAs that is typically accounted for by the biomarker considered (*c*) is 0.27 (Volkman et al. 1989).

The rate of ^{13}C loss from sediment organic matter was determined by fitting multi-*G* models to the data (Westrich and Berner 1984), which assume that sediment organic matter consists of multiple fractions (*G*) that degrade exponentially at different rates. The model used was as follows:

$$G_T(t) = G_1[\exp(-k_1 t)] + G_2[\exp(-k_2 t)] + G_{NR} \quad (1)$$

where G_T is the incorporation of ^{13}C into sediment organic matter, t is time, G_1, G_2 and G_{NR} represent the initial incorporation of ^{13}C into highly reactive, less reactive, and nonreactive (over the experimental timescale) fractions, and k_1 and k_2 are first-order decay constants.

Separate models were generated for two replicate CT and ZC plots, allowing calculation of means and standard errors for model parameters.

Total ^{13}C transfer into water column DOC and DIC was calculated for the beginning and end of the dark incubation period and for the end of the light period as the product of excess ^{13}C in DOC or DIC (fraction ^{13}C in labeled sample – fraction ^{13}C in equivalent control), core volume and concentration of DOC or DIC. The total flux of excess ^{13}C in DOC or DIC during dark or light incubation was then calculated as follows:

$$\text{Excess } ^{13}C \text{ flux} = \text{Excess } ^{13}C_{start} - \text{Excess } ^{13}C_{end} / SA / t \qquad (2)$$

where excess $^{13}C_{start}$ and excess $^{13}C_{end}$ represent the beginning and end of the incubation period, SA is the sediment surface area within a core, and t represents hours of incubation.

To account for the addition of different quantities of ^{13}C added to CT and ZC plots, all excess ^{13}C data was converted to a percentage of the ^{13}C remaining in sediments 1 d after detritus addition (following loss due to resuspension during initial site inundation), allowing comparison of the two treatment types.

Data analysis

Uptake rates of ^{13}C into *C. taxifolia* and *Z. capricorni* were determined from the linear equation that best (pog 10 data for assimilation of ^{13}C by macrophytes during isotope labeling in the laboratory. The effect of the experimental procedure on ^{13}C content of fauna, bacteria, MPB and sediment organic matter pools and on fluxes of ^{13}C in DOC and DIC was determined by comparing data collected for C and PC plots. Two-way analyses of variance (ANOVAs) determined if there was a significant effect of treatment or time or an interacting effect of time and treatment on these values ($\alpha = 0.05$). Two-way ANOVAs with factors of time and treatment were also used to compare data from CT and ZC plots. Where Levene's test returned a significant p-value, data was transformed (log (x+1)) prior to analysis to improve homogeneity of variances. Where there were significant effects, post-hoc Tukey tests indicated

which levels differed. Significant interactions were investigated using separate one-way ANOVAs for CT and ZC plots and separate one-way ANOVAs for each time.

Results

The detritus addition procedure did not significantly affect ^{13}C in sediment benthic compartments or fluxes of $DO^{13}C$ and $DI^{13}C$ ($p > 0.05$).

Approximately 5% of the ^{13}C added to sediments as *C. taxifolia* (CT plots) and *Z. capricorni* detritus (ZC plots) remained after 1 d (~5441 and ~2409 μmol ^{13}C m^{-2}, respectively) with the remainder most likely lost via re-suspension in the high energy environment (i.e., wind and boat waves and tidal flows). These were the assumed starting quantities for ^{13}C in this study. Incorporation of ^{13}C was expressed as a percentage of these quantities (%^{13}C).

Sediment compartments

There was a significant temporal difference in ^{13}C incorporation into sediment organic carbon (Table 1), but no significant difference between ZC and CT plots ($p > 0.05$) (Fig. 1A). However, multi-*G* modeling indicated that the loss of ^{13}C from sediment organic carbon was fastest for ZC plots. The highly reactive fraction (G_1) in ZC plots accounted for a similar proportion of sediment organic carbon (83.84 ± 9.14%) but degraded at a faster rate (0.31 ± 0.01 d^{-1}) than in CT plots (87.58 ± 3.17%, 0.15 ± 0.03 d^{-1}). The less reactive fraction (G_2) accounted for a higher proportion of sediment organic carbon and degraded faster in ZC plots (14.46 ± 10.14%, 0.26 ± 0.08 d^{-1}) than in CT plots (7.12 ± 1.47%, 0.13 ± 0.06 d^{-1}). The non-reactive fraction (G_{NR}) represented 1.70 ± 1.00% and 5.30 ± 4.64%, respectively, of organic carbon in ZC and CT plots.

MPB and bacteria accounted for 22.22% (1752.96 mmol C m^{-2}) and 1.07% (84.25 mmol C m^{-2}) of sediment organic carbon, respectively. There was greater incorporation of ^{13}C

into MPB than bacteria, reflecting this difference in biomass. There was significantly greater ^{13}C incorporation into MPB in ZC plots than in CT plots (Table 1) and, regardless of treatment type, ^{13}C incorporation varied among times (Table 1, Fig. 1C). For bacteria, ^{13}C incorporation was also significantly greater in ZC plots than in CT plots (Table 1), and incorporation of ^{13}C varied significantly among sampling periods regardless of treatment type (Table 1; Fig. 1D).

Macrophytes and fauna

C. taxifolia had a lower molar C:N ratio (13.78 ± 0.41) than *Z. capricorni* (20.91 ± 0.81). The rate of ^{13}C uptake into *C. taxifolia* during isotope labeling in the laboratory (Regression: 8.4 mmol ^{13}C mol C^{-1} d^{-1}, R^2 = 0.99) was more than double that of *Z. capricorni* (3.9 mmol ^{13}C mol C^{-1} d^{-1}, R^2 = 0.97) (Fig. 2).

There was evidence of ^{13}C uptake into live *C. taxifolia* and *Z. capricorni* in CT and ZC plots. For both macrophytes, ^{13}C incorporation (%^{13}C) was greater in ZC plots than in CT plots (Table 1), although this was less pronounced for *C. taxifolia* (Fig. 1E). For *C. taxifolia*, on average, ^{13}C incorporation in CT plots was 61.68% of that in ZC plots, whereas for *Z. capricorni*, ^{13}C incorporation in CT plots was only 27.95% of that in ZC plots. There was no significant effect of time for *C. taxifolia*, but for *Z.* = 0. *capricorni* there was significantly more ^{13}C in plants at the first sampling time than at all other times (Table 1; Fig. 1F).

Due to a lack of replicates across sampling periods, for mud whelks (*Velacumantus australis*), a one-way ANOVA compared ^{13}C incorporation in CT and ZC plots for samples pooled across times. *V. australis* in ZC plots incorporated significantly more ^{13}C than in CT plots (Table 1; Fig. 1B). The ^{13}C incorporation in CT plots was 16.30% of that in ZC plots.

Fluxes of DOC and DIC

The only significant difference in fluxes of DO^{13}C and DI^{13}C between CT and ZC plots was for light fluxes of DOC at day 5, when there was a greater flux of DO^{13}C from CT plots

than from ZC plots (Interaction, with effect of treatment on day 5; Table 1; Fig. 3B). There was no dark production of $DO^{13}C$ from ZC plots on day 5, compared to a flux of 0.42 ± 0.27% ^{13}C from CT plots, but this difference was not significant. At all other times, light and dark fluxes of DOC and DIC from CT and ZC plots were similar ($p > 0.05$).

There was no significant temporal variability in dark $DO^{13}C$ fluxes, light $DO^{13}C$ fluxes from ZC plots, or light $DI^{13}C$ fluxes (Fig. 3). There was, however significant temporal variation in light $DO^{13}C$ fluxes from CT plots, with significantly greater fluxes on day 5 than at any other time (Interaction, with effect of time for CT; Table 1; Fig. 3B), and temporal variation in dark fluxes of $DI^{13}C$ regardless of treatment type (Table 1; Fig. 3C), with greater fluxes on days 5 and 8 than on days 21 and 31, and fluxes on day 13 intermediate.

Discussion

Although macroalgal detritus is typically rapidly processed and transferred to heterotrophs (Mann 1988), the current study suggests that this is not the case for *C. taxifolia*. Given that the first measurement of carbon flux from sediments was taken 5 d after addition of detritus, it is possible that some initial demineralization of *C. taxifolia* detritus was missed. However, the similar loss of carbon from sediments over 1 d following detritus addition, and the lack of carbon transfer to biota by the time the first samples were taken, suggest that this is unlikely. Rather, over the timescale of this experiment, carbon from seagrass detritus was removed from sediments more rapidly (albeit not significantly so) than that from macroalgae, and there was significantly greater uptake of seagrass-derived carbon than macroalgae-derived carbon for all of the compartments studied. Such discrimination hints at the possibility that *C. taxifolia* may alter carbon cycling in invaded habitats, contributing to its invasive success and effects on biota.

In Moreton Bay, where the current study was done, it has been suggested that *C. taxifolia* colonizes sediments following seagrass loss rather than directly replacing seagrass (Burfeind and Udy 2009). While healthy, dense seagrass beds are relatively resistant to invasion (Jaubert et al. 1999), seagrass and *C. taxifolia* can co-occur, as was the case at the site we studied. Initial colonization by *C. taxifolia* is likely to be facilitated by seagrass deterioration due to existing stressors (e.g., nutrient over-enrichment, disturbance, and light limitation), but further invasion may be facilitated by a variety of mechanisms (Ceccherelli and Cinelli 1997).

Previous studies have suggested a number of mechanisms that may assist *C. taxifolia* invasion, including allelopathy assisted by the toxic caulerpenyne (Ferrer et al. 1997), and the creation of sediment conditions conducive to production of sulfide, which is toxic to seagrass (Holmer et al. 2009). Here we propose that the ability of *C. taxifolia* to utilize carbon derived from its own detritus is a further mechanism that may assist its invasion.

Seagrass productivity can be carbon limited (Durako 1993). Although plants typically acquire carbon through DIC produced by respiration and organic matter demineralization, DOC leached from organic matter or released via sloppy feeding and from plant roots can also be a source (Smith and Penhale 1980). Given that fluxes of DOC and DIC to the water column represent the balance between production and utilization within the sediment, the similarity in light and dark fluxes of $DI^{13}C$ from CT and ZC plots in the current study suggests that there was similar net production and utilization of DIC from macroalgae and seagrass detritus. The similarity in DIC fluxes most likely reflects differences in DOC production and/or utilization underpinning the differences in carbon uptake observed for macrophytes in ZC and CT plots. $DO^{13}C$ fluxes from CT plots that were elevated above those from ZC plots at day 5, combined with the lower uptake of macroalgae-derived detritus by macrophytes and heterotrophs, indicate that there was lower use of DOC released from macroalgae detritus than from seagrass detritus. This is likely to reflect differences in the composition of the DOC pool, which may

contain labile and refractory components that can be selectively utilized (Moran and Hodson 1989).

Both *C. taxifolia* and *Z. capricorni* incorporated carbon derived from seagrass detritus in preference to that from macroalgae detritus. However, this preference was more pronounced for *Z. capricorni*, which derived almost no carbon from macroalgae, suggesting that *C. taxifolia* is better able to use carbon from its own detritus than is *Z. capricorni*. This most likely relates to the presence of compounds in DOC derived from *C. taxifolia* that are unable to be utilized by *Z. capricorni*. Given the ability of the secondary metabolite caulerpenyne to restrict the productivity of macrophytes (Ferrer et al. 1997), this appears to be a likely candidate. Caulerpenyne is carbon-rich and may leach from *C. taxifolia* detritus, providing an additional source of carbon available primarily to *C. taxifolia*. This is particularly the case early in the experiment, when leaching is a major pathway for DOC release (Casteldelli et al. 2003). Later in the study, the difference in uptake of carbon from macroalgae and seagrass detritus was less pronounced, presumably as macrophytes obtained carbon from DIC and DOC pools within the sediment.

Following invasion, *C. taxifolia* is expected to make a considerable contribution to sediment organic matter through its detritus, particularly as rates of direct grazing are low (Gollan and Wright 2006). There has previously been little investigation of the effect of *C. taxifolia* detritus in invaded areas. Regardless of the underlying process, the current study suggests that input of *C. taxifolia* detritus into sediment will give *C. taxifolia* a competitive advantage over seagrass, as it is better able to access carbon derived from its own detritus. As invasion progresses, this effect would be magnified. This may particularly be the case where high nutrient concentrations are present, exacerbating carbon limitation of macrophyte production. In this case, the combined effect of competitive advantage of *C. taxifolia* in terms of carbon, and potentially nutrients (Ceccherelli and Cinelli 1997) would enhance invasive success.

Mechanisms for effects of C. taxifolia *invasion on biota*

Numerous studies have demonstrated that some species of biota are less abundant in *C. taxifolia* than in seagrass (Burfeind et al. 2009; McKinnon et al. 2009). Although this can relate to physical differences in habitat structure (Longepierre et al. 2005), differences in availability of sources of nutrition (carbon) for consumers are also likely to alter assemblages.

Whereas seagrass beds are recognized as a valuable source of carbon for consumers (Duarte 2002), *Caulerpa* spp. are generally unpalatable to fish and invertebrates (Erickson et al. 2006). Although some fishes and invertebrates assimilate carbon derived from *Caulerpa* spp. (Box et al. 2009; Casu et al. 2009), this can be associated with physiological effects, suggesting that ingestion is incidental in some cases (Box et al. 2009). Some species (e.g., sea slugs) have mechanisms to avoid effects of, and even exploit, algal toxins (Mollo et al. 2008), but colonization by these species may represent a change in community assemblage following *Caulerpa* spp. invasion.

We have shown that MPB, bacteria and higher consumers (whelks) prefer carbon derived from seagrass detritus to that from *C. taxifolia* detritus. As was described for macrophytes, this may be due to differences in the DOC produced from each kind of detritus. MPB and bacteria may be better able to utilize carbon derived from *Z. capricorni* due to the presence of toxic metabolites in DOC from *C. taxifolia* detritus. This preference would be reflected in the isotopic signature of higher consumers that rely on MPB and/or bacteria as a carbon source. Alternatively, higher consumers may obtain carbon directly from labeled macrophytes and/or detritus. The presence of caulerpenynes in live tissues of *C. taxifolia*, and the potential for caulerpenynes to be concentrated in detritus (Raniello et al. 2007), would result in greater uptake of carbon from *Z. capricorni*. The inability of heterotrophs to utilize carbon from *C. taxifolia* detritus, combined with reduced fitness due to physiological effects of *C. taxifolia* consumption (Box et al. 2009), may explain the reduced abundance and biomass of

biota in seagrass beds invaded by *C. taxifolia*. The ability of some species to utilize *C. taxifolia*-derived carbon would lead to differences in species assemblages.

Implications

Despite *Caulerpa* spp. having invaded large areas of many coastal systems, little was previously known of the contribution of *Caulerpa* spp. detritus to invasion success. As well as assisting *C. taxifolia* invasion, differences in the processing and utilization of carbon derived from seagrass and *C. taxifolia* detritus are likely to influence carbon cycling in invaded habitats by increasing DOC fluxes to the water column (Eyre et al. in press) and altering ecosystem trophodynamics. Although increased release of DOC from *C. taxifolia* detritus compared to *Z. capricorni* was observed only in the initial stages of the current study, where there are constant inputs of *C. taxifolia* detritus to sediments (rather than the pulsed input used in this study), this effect would be constant.

The availability of an additional carbon source for *C. taxifolia*, generated from its own detritus, represents a competitive advantage for the invasive macroalgae over existing seagrass, particularly as seagrass productivity can be carbon limited. A similar mechanism may exist for other species of invasive macroalgae, particularly those that produce toxic secondary metabolites. A combination of the mechanism we describe and those previously identified are likely to account for the invasive success of *Caulerpa* spp. in seagrass beds, particularly in the presence of existing stressors.

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Table 1: Significant results of analyses of variance (ANOVAs) looking at the effects of time, treatment, and the interaction of time and treatment on ^{13}C uptake into sediment and water column compartments following addition of ^{13}C-labeled *Caulerpa taxifolia* (treatment CT) or *Zostera capricorni* (treatment ZC) detritus. Two-way ANOVAs were used in all cases except for mud whelks, where a one-way ANOVA tested the effect of treatment only. Significant results of one-way ANOVAs used to investigate interactions are also shown. Where the effect of treatment was significant, outcomes of Tukey tests indicate which treatment had higher ^{13}C uptake. - = not applicable, df = degrees of freedom.

Compartment	Significant effect	*F*-value	df	*p*-value	Tukey result
Sediment organic carbon	time	10.297	5,19	<0.001	
Mud whelk	treatment	43.889	1,11	<0.001	ZC > CT
Microphytobenthos	treatment	6.445	1, 20	0.020	ZC > CT
	time	2.737	5,20	0.048	
Bacteria	treatment	7.521	1,18	0.013	ZC > CT
	time	3.554	5,18	0.021	
Macroalgae (*C. taxifolia*)	treatment	6.640	1,22	0.017	ZC > CT
Seagrass (*Z. capricorni*)	treatment	10.822	1,17	0.005	ZC > CT
$DO^{13}C$ dark	-				
$DO^{13}C$ light	time x treatment	12.296	4,16	<0.001	
	Day 5: treatment	20.824	1, 3	0.020	CT > ZC
	CT: time	18.404	4, 8	<0.001	
$DI^{13}C$ dark	time	10.366	4, 5	0.012	
$DI^{13}C$ light	-				

Figure legends

Figure 1: Excess ^{13}C in (A) sediment organic carbon, (B) mud whelks, (C) microphytobenthos, (D) bacteria, (E) macroalgae, and (F) seagrass over time as a % of the ^{13}C added as *Caulerpa taxifolia* (CT) and *Zostera capricorni* (ZC) detritus (mean ± SE). Lines are a visual aid, except for sediment organic carbon, where lines indicate multi-*G* models that best fit the data.

Figure 2: ^{13}C incorporation by live *C. taxifolia* and *Z. capricorni* during laboratory incubation with ^{13}C-labeled sodium bicarbonate ($NaH^{13}CO_3$, 99% ^{13}C) (mean ± SE). Lines represent linear regressions.

Figure 3: Excess ^{13}C in $DO^{13}C$ during the (A) dark and (B) light, and $DI^{13}C$ fluxes during the (C) dark and (D) light over time as a % of the ^{13}C added to sediments as *C. taxifolia* (CT) and *Z. capricorni* (ZC) detritus (mean ± SE). Lines are a visual aid.

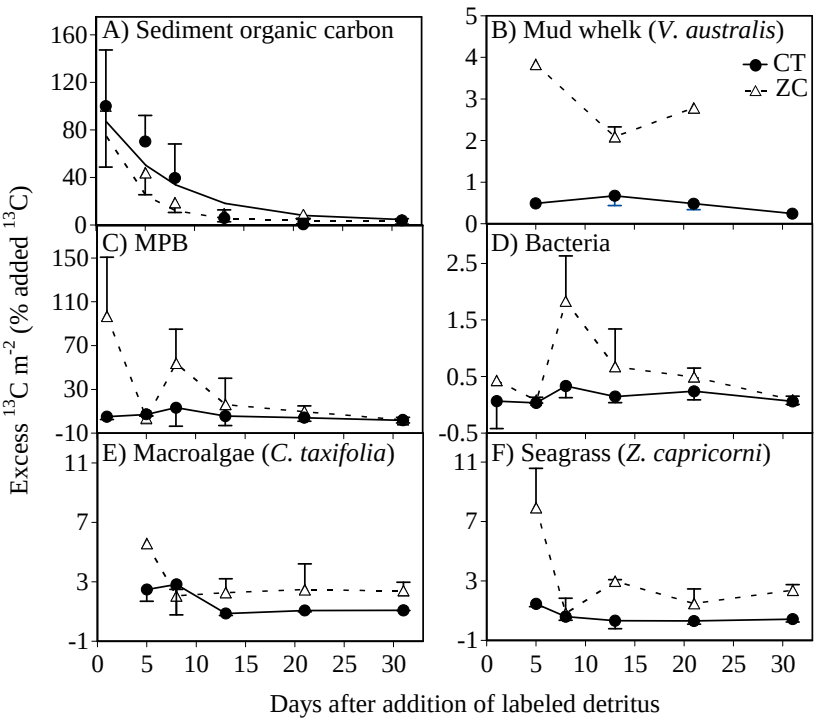
A) Sediment organic carbon
B) Mud whelk (V. australis)
CT
ZC
C) MPB
D) Bacteria
E) Macroalgae (C. taxifolia)
F) Seagrass (Z. capricorni)
Excess 13C m-2 (% added 13C)
Days after addition of labeled detritus

Fig. 1

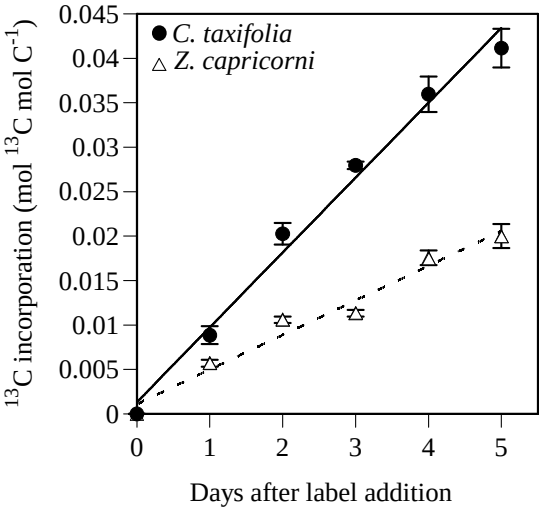
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
^{13}C incorporation (mol ^{13}C mol C^{-1})
C. taxifolia
Z. capricorni
0
1
2
3
4
5
Days after label addition

Fig. 2

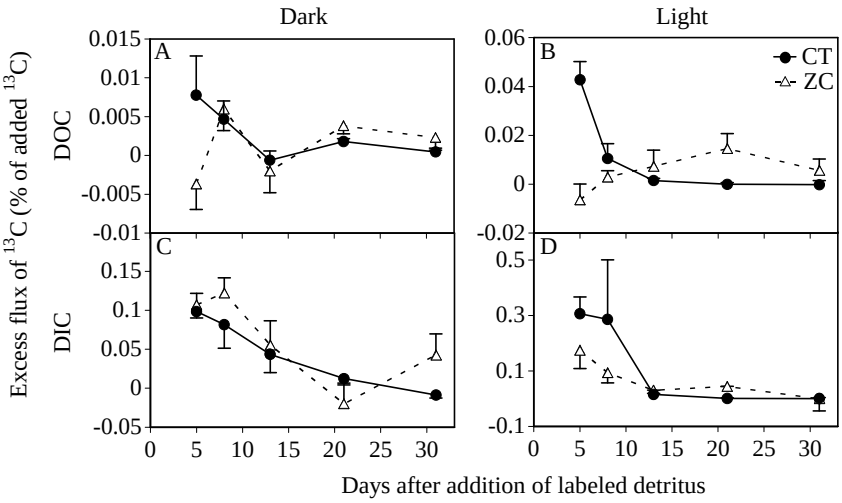
Dark
Light
A
B
C
D
CT
ZC
DOC
DIC
Excess flux of ^{13}C (% of added ^{13}C)
Days after addition of labeled detritus

Fig. 3